Explor Anim Med Res.
Vol. 15, Issue 2, 2025
DOI: 10.52635/eamr/15.2.206-218

ISSN 2277-470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Published under the CC BY-NC 4.0 license

Research Article

EARLY CARDIAC DAMAGE FOLLOWING SINGLE AND COMBINED EXPOSURE TO LITHIUM CARBONATE AND LEAD ACETATE IN RATS: A TOXICOLOGICAL PERSPECTIVE

Harsh R. Patel[1*], Urvesh D. Patel[2], Maunik D. Prajapati[1], Brijesh R. Humbal[1], Jahnavi P. Patel[1], Dhaval T. Fefar[3]

Received 24 August 2025, revised 12 October 2025

ABSTRACT: Environmental contamination by heavy metals, especially lithium (Li) and lead (Pb), is of growing concern due to their increasing industrial applications and accumulation in ecosystems. This study investigated the cardiotoxic and systemic effects of individual and combined oral exposure to lithium carbonate and lead acetate in forty-eight adult male rats over a 28-day period. Animals were assigned to four groups: control, Li (20 mg/kg), Pb (20 mg/kg), and Li + Pb (20 mg each/kg). All toxicity groups exhibited clinical signs such as dullness, dehydration, diarrhea, hair loss, and foul-smelling feces. Electrocardiographic (ECG) abnormalities, including shortening of QRS duration, changes in P- and T-wave amplitudes, and sporadic atrial flutter in a few animals, suggest the presence of cardiac dysrhythmia in the treatment groups. Although no significant changes in heart weights or indices were observed, the Li + Pb group showed significantly elevated levels of malondialdehyde (MDA) and nitric oxide (NO), alongside decreased total antioxidant capacity (TAC), suggesting oxidative stress. No significant change was observed in the antioxidant enzyme (SOD, CAT, and GSH) level in the treatment groups. Molecular analysis revealed downregulation of *nrf2* and *bcl-2*, and upregulation of *bax* in the Li + Pb group, suggesting increased apoptosis and reduced cell survival. Interestingly, Pb alone upregulated *bcl-2* more than Li, indicating a differential stress response. Histological examination showed no overt structural damage to myocardial tissue. These findings indicate that co-exposure to Li and Pb exerts synergistic cardiotoxic effects primarily via oxidative stress and apoptosis, warranting attention to combined environmental exposures in public health assessments.

Keywords: Cardiotoxicity, Electrocardiography, Histopathology, Lead acetate, Lithium carbonate, mRNA expression, Oxidative stress.

INTRODUCTION

Heavy metal pollution has increased with the expansion of mining, agriculture, waste management, and the automobile industry. These persistent metals bioaccumulate in the food chain [1] and leach into soil and water [2], posing serious health risks - especially cadmium, mercury, lead, and lithium, whose levels are rising with electric technologies [3, 4, 5]. Heavy metals and therapeutic agents may interact to increase cardiac toxicity. Because heavy metals induce oxidative stress, evaluating their effects on antioxidant defenses is essential for risk mitigation [3].

Lithium is naturally present in the environment, occurring in soil at concentrations up to 200 µg/g and in surface water ranging from 1000 to 10,000 µg/l, with certain Chilean rivers containing as much as 5000 µg/l [6]. It is also found in various plants, algae, and marine organisms [7], while the average concentration in the earth's crust is approximately 50–65 mg/kg [8]. Soil levels range from 10–100 mg/kg in the U.S. and

[1]*Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India.*
[2]*Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Himmatnagar, Gujarat, India.*
[3]*Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat, India.*
**Corresponding author. e-mail: patelharsh2708@gmail.com*

10–50 mg/kg in Russia [9]. Lithium has been measured at 1173 µg/l in the Pacific and 160 µg/l in the Indian Ocean [6], with drinking water in parts of Texas reaching 78000 µg/l [10]. The growing use of lithium-ion batteries in electric and hybrid vehicles is driving lithium market growth [11]. Recycling processes like leaching recover lithium carbonate from spent batteries [12], but inadequate recycling and disposal remain key sources of environmental lithium pollution [13].

Lithium occurs naturally in soil (up to 200 µg/g) and surface waters (1000–10,000 µg/l), with some Chilean rivers reaching 5000 µg/l [6]. It is present in plants, algae, and marine organisms [7], and averages 50–65 mg/kg in the earth's crust [8]. Soil concentrations range from 10–100 mg/kg in the U.S. and 10–50 mg/kg in Russia [9]. Reported levels include 1173 µg/l in the Pacific, 160 µg/l in the Indian Ocean [6], and up to 78,000 µg/l in Texas drinking water [10].

Excess lithium accumulation promotes oxidative stress and apoptosis, triggering cardiac inflammation with elevated cytokine production [11, 12] and increased TNF, which worsens cardiovascular injury [13]. Lithium carbonate disrupts ROS–antioxidant balance, driving lipid peroxidation and mitochondrial ETC impairment, causing electron leakage and superoxide generation; elevated MDA in treated rats confirms this damage [14, 15, 16]. Antioxidant enzymes such as SOD, CAT, and GPx are altered, intensifying cellular injury [17, 18]. Lithium also activates mitochondrial apoptosis via cytochrome-C release and altered bcl-2/bax expression, increasing susceptibility to cell death [19].

Lead (Pb) exposure poses major cardiovascular risks by driving oxidative stress, inflammation, and endothelial dysfunction, contributing to hypertension and heart disease [20]. Despite declining overall Pb levels, children and industrial workers remain highly vulnerable [21]. Persistent environmental sources—including batteries, fuels, and paints—sustain exposure, making Pb a long-term cardiovascular threat, particularly in developing regions with weak regulatory control [22, 23].

The Indian Standard Drinking Water Specification (1991) limits lead to 50 µg/l, while WHO sets it at 25 µg/l. However, much higher levels were reported in Gujarat lakes - 4500 µg/l in Vastral Lake and 1800 µg/l in Sarkhej Lake [24, 25, 26]. Soil Pb can reach 15–40 mg/kg in highly contaminated regions [27], while U.S. surface and groundwater levels are ~5 and 30 µg/l, respectively. After ingestion, Pb is absorbed via the gastrointestinal tract, circulates systemically, and affects multiple organs including the heart [28], elevating blood pressure and impairing cardiac function. Pb inhibits key antioxidant enzymes (GR, GPx) and increases hemoglobin auto-oxidation [29, 30, 31], while damaging cardiac cell membranes and exacerbating oxidative injury [32, 33]. It binds –SH, $–NH_2$, and –OH groups and competes with calcium in Ca-activated proteins, disrupting cardiac function [34], increasing ROS, lipid peroxidation, and tissue damage [35]. Co-exposure studies (Pb+Li, Li+Mn) demonstrate synergistic toxicity in mouse brain stem cells over 28 days [36], and greater stress responses in *Mytilus galloprovincialis* compared with single-metal exposure [37].

The *nrf2* is a key regulator of cardiac antioxidant defense; normally bound to *Keap1* in the cytoplasm, it translocates to the nucleus under ROS stress to activate antioxidant genes such as HO-1 [38, 39, 40]. The *Bax*, a pro-apoptotic *bcl-2* family member, promotes mitochondrial dysfunction and cardiomyocyte apoptosis. However, the combined effects of Li and Pb on *nrf2*- and *bax*-mediated signaling remain unclear. Heavy metals cause genotoxicity primarily via excess free-radical generation leading to DNA strand breakage and functional impairment if unrepaired [41]. Pb consistently enhances apoptosis by increasing *bax*, *caspase-3*, and *nrf2* while reducing *bcl-2* [42, 43, 44], whereas Li shows variable effects on apoptotic regulation.

This study aims to investigate the combined effects of lithium and lead exposure on electrocardiography, cardiac oxidative stress markers, the expression of genes related to oxidative stress and apoptosis, and histopathology in cardiac tissue. To better understand the cardiotoxic mechanisms and interactions of these metals, the present study is designed to evaluate their individual and combined impact through daily oral administration in rats over 28 days.

MATERIALS AND METHODS

Test compounds

Lithium carbonate (≥99.5% purity, CAS No. - 554-13-2) and lead acetate (≥97.00% purity, CAS No. - 6080-56-4) were purchased from Himedia, India. Stock solutions of lithium carbonate and lead acetate were prepared in Milli-Q water at a concentration of 8 mg/ml, and diluted to achieve the required doses. Accurate weighing of each powder was done using a calibrated weighing balance (Model: MS 204S/A01, Mettler Toledo, US). These stock solutions were used further to get the desired concentration.

Experimental animals

The study was conducted on 48 male Wistar rats (7-8 weeks old) obtained from Zydus Research Centre,

Ahmedabad, Gujarat. The protocol was approved by the IAEC (Protocol No. KU-JVC-IAEC-SA-123-23, dated December 16, 2023), and followed CCSEA guidelines (2003). Rats were housed in polypropylene cages with stainless steel grills and corn cob bedding, and were changed twice weekly. Standard conditions were maintained: 12-hour light/dark cycle, temperature 23.19 ± 0.13 °C to 25.76 ± 0.13 °C, and humidity 48 ± 0.53% to 65 ± 0.74%. All animals had ad libitum access to standard feed (VRK Nutritional Solutions, Maharashtra) and filtered RO water via 300 ml polypropylene bottles. Rats were acclimatized for 7 days and individually marked on the tail with a black marker. A Completely Randomized Design (CRD) was followed with four treatment groups (n=12), randomized by body weight within a 5% variation.

Exposure

Rats in the control group were administered with R.O. water in the volume range of 0.75 to 1.12 ml based on the body weight of each animal. Lithium carbonate and lead acetate were individually administered at a dose rate of 20 mg/kg BW daily for 28 days to rats of the Li and Pb groups, respectively. The rats in the Li + Pb group were administered lithium carbonate and lead acetate at both dose rates at 20 mg each/kg BW daily for 28 days. The selected dose levels for both single and combined administration were based on previous studies that demonstrated observable toxicological effects without causing overt mortality, thus making them suitable for sub-chronic exposure evaluations [45, 46, 47]. Looking to the aim of comparison of toxic effects of both compounds at same dose, 20 mg/kg dose was chosen for both compounds in this study.

Clinical manifestation

Throughout the treatment period, all rats were monitored twice daily for any clinical signs or any abnormalities. Observations like hair loss, diarrhoea, dullness, dehydration, and foul smell were graded from 0 to 3 scale (0: None; +: Mild; ++: Moderate; +++: Severe) [48].

Electrocardiography

An Electrocardiograph (ECG) machine (SSECG-300V, Nainital, Uttarakhand, India) was used to record the heart's electrical activity, using a standard lead II configuration tailored for rats. To minimize stress and reduce movement artifacts, light anesthesia (Thiopental sodium 40 mg/kg, I/P) was administered. Thiopental may transiently depress heart rate and alter cardiac conduction, which was considered while interpreting ECG parameters. Needle electrodes, compatible with small animal models, were applied to the forelimbs, hindlimbs, and thoracic area, with conductive gel on each electrode to enhance signal quality. The ECG was recorded at a speed of 50 mm/second, with the amplitude set to 10 mm/mV for clear visualization of the waveforms. Signals were processed with a 50–75 Hz bandpass filter, and measurements included P duration, QRS duration, T duration, R interval, P wave height, QRS complex, T wave height, and heart rate, which were recorded in six animals from each experimental group.

Evaluation of heart weight, index

The weights of the heart recorded and the heart indices were determined by dividing the organ weight by the final body weight and multiplying by 100.

Evaluation of oxidative stress parameters in the cardiac tissue

Heart tissue samples were collected individually from six rats in each group (n = 6) and collected in Tris–EDTA buffer having pH 8.2-8.5 (100 mg/ml) for evaluation of superoxide dismutase (SOD) activity [49], in phosphate-buffered saline (PBS, pH 7.5) for measuring catalase (CAT) [50], total antioxidant capacity (TAC; EZAssay™ Antioxidant Activity Estimation Kit (CUPRAC)), nitric oxide (NO; EZAssay™ Nitric Oxide Estimation Kit), lipid peroxidation (MDA; EZAssay™ TBARS Estimation Kit for Lipid Peroxidation), glutathione reductase (GSH) [51], and protein levels [52]. Samples collected in sterile K_3EDTA tubes were used for the preparation of blood lysates for CAT [53] and GSH assessments [51]. Samples in anticoagulant-free vials were allowed to clot at room temperature, after which serum was separated by centrifuging (Eppendorf 5430 R, Germany) at 10,000 g for 10 minutes. The serum was then stored at -80°C for subsequent oxidative stress analyses, including measurements of SOD, MDA, TAC, and NO levels which were done within 2 days. All tissue samples in the buffer were centrifuged at 12,000 g for 5 minutes, except for SOD, which was centrifuged for 40 minutes at the same speed. The resulting supernatants were used for estimating various antioxidant enzymes.

Evaluation of gene expression in the cardiac tissue

The heart samples (n=6) from rats of each group were collected in a DNase and RNase-free storage vial with RNA later solution (approximately 10 times the tissue volume) for ribonucleic acid (RNA) isolation and

complementary DNA (cDNA) synthesis to determine the mRNA expression levels of *nrf2*, *bax* and *bcl2* genes. The high-quality total RNA was isolated as per the standard protocol [54]. The RNA samples having a 260/280 ratio of ~2.0 were treated with DNA-freeTM Kit for removal of genomic DNA contamination, and cDNA was synthesized using the first strand cDNA synthesis kit. The *β-actin*, *nrf2*, *bax,* and *bcl2* gene-specific primers (Table 1) were used for amplification in a real-time PCR system (LightCycler® 480 II, Roche, Basel, Switzerland) using Maxima SYBR Green/ROX qPCR master mix. The PCR-amplified products were confirmed with agarose gel (2.4%) electrophoresis. The β-actin gene was taken as a reference gene. The fold expression changes were calculated by the $2^{-\Delta\Delta Ct}$ method [55].

Histopathological examination of the cardiac tissue

End of the experiment, 24 rats (six from each group) was used for histopathological examination of the heart. Formalin-fixed tissues were embedded in paraffin and processed as per standard protocols. Sections were cut to a thickness of 5 µm using a semi-motorized rotary microtome (Leica Biosystems, Germany) and stained with hematoxylin and eosin (H and E) stain [56]. The stained slides were examined under a Zeiss Primo Star microscope equipped with a microscopic camera (Zeiss Axiocam ERC). Histological images were captured using Carl Zeiss Zen (Blue Edition) software.

Statistical analysis

Statistical analyses of all data were carried out using GraphPad Prism 9.0. The Kolmogorov-Smirnov test was used to evaluate the normality of data, along with Bartlett's test to confirm the equal variance. Data with normal distribution and homogeneous variance were analyzed by parametric one-way analysis of variance (ANOVA) followed by Tukey's HSD test. The data that didn't have either a normal distribution or equal variances were analyzed by the Kruskal-Wallis test, followed by Dunn's test. The value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Clinical manifestation

The mortality rate was not recorded in any of the toxicity groups throughout the study. Symptoms of toxicity observed in the toxicity groups were hair loss, diarrhea, dullness, dehydration, and foul-smelling faeces. Hair loss and diarrhea were uniform in the Li and Pb-treated groups, but more pronounced in the Li + Pb-treated group. Dehydration occurred more frequently in the Pb-treated and Li + Pb-treated groups. Diarrhea was more common in the Li-treated group.

Alopecia, diarrhea, and dehydration in treated rats indicate systemic toxicity, most severe in the co-exposed group. Lithium may induce polyuria, dehydration, and electrolyte imbalance, while lead impairs gastrointestinal function through mucosal irritation, altered motility, and microbiome disruption. More pronounced dehydration in Pb and Li+Pb groups suggests a stronger gastrointestinal effect of lead. Diarrhea, seen particularly in the Li group, likely reflects lithium-induced mucosal irritation during prolonged oral exposure. Similar diarrhea and polydipsia occur in humans receiving long-term lithium therapy, often linked to body damage [57, 58].

Electrocardiograph

Any treatment did not significantly alter the duration of P, T, and R waves. However, the duration of the QRS complex (ventricular depolarization) was significantly lower in Li- and Pb-treated rats, but not in the Li + Pb co-exposed group. The height of the P wave (atrial depolarization) was significantly reduced in the Pb and Li + Pb-treated groups compared to the control group. The T-wave height, reflecting ventricular repolarization, was significantly decreased in the Li-treated group, while no significant change was observed in the Pb-treated group compared to the control, and significantly reduced in the Li + Pb co-exposed group, indicating differential effects of individual and combined exposures. Heart rates remained similar across all toxicity groups, and QRS complex height showed no significant changes compared to the control group. ECG recordings revealed atrial flutter in a few animals from each of the toxicity groups, rather than in all animals. It was not common finding in all the six animals in a treated group, likely due to limited conduction of atrial impulses through the AV node and compensatory autonomic regulation maintaining the ventricular rate. Overall, Li, Pb, and Li + Pb affected ECG waves and segments in a parameter-specific manner, with QRS duration showing a reversal effect in the co-exposure group compared to individual compounds, suggesting a toxicological interaction between the two agents. ECG recording parameters and ECG abnormalities in rats exposed to Li, Pb, and Li +Pb are illustrated in Table 2 and Fig. 1.

Previous studies support our findings of ECG abnormalities following Li and Pb exposure. Lithium-treated rats showed ischemic changes such as depressed ST segments and reduced R-wave amplitude [59],

Table 1. Primers used for real-time PCR amplification of target genes.

Name of the gene	Primer sequence (5'-3')	Annealing temperature (°C)	Product size (bp)	Accession Number	Reference
Nrf$_2$	FP - TGTAGATGACCATGAGTCGC RP - TCCTGCCAAACTTGCTCCAT	58	159	NM_031789.2	[72]
Bax	FP - GGGCCTTTTTGCTACAGGGT RP - TTCTTGGTGGATGCGTCCTG	58	106	NM_017059.2	[71]
Bcl-2	FP - ACTCTTCAGGGATGGGGTGA RP - TGACATCTCCCTGTTGACGC	58	94	NM_016993	[71]
β-actin	FP - AACCCTAAGGCCAACCGTG RP - TACGTACATGGCTGGGGTGT	58	72	KJ_696744.1	[73]

Table 2. ECG in male rats exposed to lithium carbonate and lead acetate.

ECG recording	Treatment groups (Mean ± SE; n=6)			
	Control	Li	Pb	Li + Pb
P duration (Sec)	0.145 ± 0.008	0.133 ± 0.003	0.138 ± 0.004	0.130 ± 0.004
QRS duration (Sec)	0.022 ± 0.002^{a}	0.012 ± 0.002^{bc}	0.010 ± 0.000^{c}	0.017 ± 0.002^{ab}
T duration (Sec)	0.137 ± 0.006	0.132 ± 0.003	0.132 ± 0.005	0.130 ± 0.004
R interval (Sec)	0.132 ± 0.007	0.130 ± 0.004	0.132 ± 0.004	0.128 ± 0.004
P wave height (mm)	1.333 ± 0.211^{a}	0.917 ± 0.083^{ab}	0.750 ± 0.112^{b}	0.667 ± 0.105^{b}
QRS complex (mm)	6.083 ± 0.239	6.250 ± 0.602	4.833 ± 0.543	4.667 ± 0.558
T wave height (mm)	2.583 ± 0.352^{a}	1.417 ± 0.154^{b}	1.500 ± 0.258^{ab}	1.583 ± 0.352^{ab}
Heart rate (bpm)	222.333 ± 9.102	225.333 ± 5.340	225.833 ± 7.300	228.667 ± 6.697

* Values with different superscripts in a row were significantly different ($p<0.05$).

Table 3. Heart weight (g) in male rats exposed to lithium carbonate and lead acetate.

Organ weight (g)	Treatment groups (Mean ± SE; n=6)			
	Control	Li	Pb	Li + Pb
Heart	1.06 ± 0.07	0.99 ± 0.06	1.02 ± 0.02	1.05 ± 0.01

* Values with different superscripts in a row were significantly different ($p<0.05$).

Table 4. Organ index (%) in male rats exposed to lithium carbonate and lead acetate.

Organ index (%)	Treatment groups (Mean ± SE; n=6)			
	Control	Li	Pb	Li + Pb
Heart	0.273 ± 0.015	0.261 ± 0.015	0.269 ± 0.012	0.283 ± 0.007

* Values with different superscripts in a row were significantly different ($p<0.05$).

which in our study appeared functionally without corresponding histopathology—likely because functional changes precede structural injury during 28-day exposure. Reduced heart rate has been reported with lithium carbonate in mice [60]. Lead acetate increased T-wave duration without affecting QRS intervals

Table 5. Oxidative stress parameters in the heart of male rats exposed to lithium carbonate and lead acetate.

Parameters	Treatment groups (Mean ± SE; n=6)			
	Control	Li	Pb	Li + Pb
SOD activity (Unit/mg of protein)	2.70 ± 0.61	2.38 ± 0.17	2.58 ± 0.71	2.05 ± 0.29
CAT activity (Unit/mg of protein)	0.481 ± 0.158	0.441 ± 0.133	0.415 ± 0.085	0.323 ± 0.075
GSH level (µg/mg of protein)	16.16 ± 1.50	14.30 ± 1.43	14.65 ± 1.64	9.90 ± 2.56
MDA level (µM)	2.80 ± 0.39^{c}	5.28 ± 0.66^{b}	5.92 ± 0.70^{ab}	7.93 ± 0.65^{a}
TAC (µM)	285.24 ± 8.62^{a}	219.05 ± 7.79^{b}	191.43 ± 16.85^{b}	101.67 ± 10.71^{c}
NO level (µM)	22.60 ± 3.84^{a}	30.62 ± 3.89^{ab}	32.11 ± 4.03^{ab}	38.09 ± 3.68^{b}

* Values with different superscripts in a row were significantly different ($p<0.05$).

Table 6. The *nrf*$_2$, *bax*, and *bcl-2* mRNA expression (fold changes; $2^{-\Delta\Delta Ct}$) in the heart of male rats under the different treatments.

mRNA expression	Treatment groups	Fold changes ($2^{-\Delta\Delta Ct}$)	± S.E.M	p value	Changes in expression
nrf$_2$	Li	1.63	0.12	0.1509	Increased
	Pb	1.65	0.24	0.1386	Increased
	Li + Pb	1.15	0.33	0.9204	Decreased
bax	Li	1.05	0.37	0.9980	Decreased
	Pb	1.48	0.28	0.4789	Decreased
	Li + Pb	1.56	0.24	0.3693	Increased
bcl-2	Li	1.90	0.27	0.0663	Increased
	Pb	4.54	0.30	<0.0001	Increased
	Li + Pb	1.14	0.20	0.9515	Decreased

* Values with different superscripts in a row were significantly different ($p<0.05$).

[61]; while one study noted no consistent heart-rate changes [62], others reported tachycardia [63, 61], and another found no change vs. control [64]. Our ECG results - altered P wave, QRS complex, and T wave - align with these reports, indicating direct effects of Li and Pb on cardiac electrophysiology. Lead disrupts calcium and potassium flux, causing conduction delay and arrhythmias [65], while lithium affects excitability by inhibiting Na^+/K^+-ATPase and disturbing Na^+/K^+ homeostasis [66]. Atrial flutter in the co-exposed group suggests electrophysiological disturbance, possibly through oxidative or ischemic atrial remodeling. The lack of matching histopathology implies early or reversible functional involvement. Similar abnormalities under heavy-metal exposure have been reported in sub-acute Pb studies [59], and Li+Pb co-exposure may potentiate these effects, with altered QRS and T waves possibly linked to mitochondrial-mediated shifts in membrane excitability or gap-junction remodeling [34].

Heart weight and index

No significant changes were observed in the heart weight in any toxicity groups compared to the control group. The effects of daily oral administration of Li, Pb, and Li + Pb for 28 days on heart weight (g) and index in rats are depicted in Table 3 and 4. Heart indices remained unchanged across groups. Overall, heart index trends aligned with heart weight findings.

The heart-to-body weight ratio (cardiac index) was significantly higher in the Li + Pb group compared to controls and individual exposure groups, despite no significant change in absolute heart weight. This elevation likely reflects reduced body weight—particularly in Pb and Li + Pb groups—rather than true cardiac hypertrophy. Thus, the increased cardiac index may represent an early adaptive or compensatory response to toxic stress rather than structural enlargement [67, 38].

Oxidative stress parameters of the heart

In the heart, no significant change was observed in the activities of SOD and CAT, as well as in the level of GSH, in all toxicity groups compared to the control group. However, the MDA level was significantly higher in the Li, Pb, and Li + Pb-treated groups. Compared to the Li-treated group, the Li + Pb group exhibited a significantly higher value for MDA. The TAC was significantly and chronologically lower in the Li, Pb, and Li + Pb-treated groups. Compared to the Li and Pb-treated group, the Li + Pb-treated group exhibited a significantly lower TAC level. No significant change was observed in the NO level in the Li- and Pb-treated groups compared to the control group, whereas a significant increase was observed in the Li + Pb co-exposed group. The oxidative stress markers in the heart of rats following 28 days of Li, Pb, and Li + Pb administration are depicted in Table 5.

Oxidative stress appeared as a key pathological mechanism. The Li + Pb group showed markedly elevated MDA and NO, along with reduced TAC, indicating heightened lipid peroxidation, nitrosative stress, and redox imbalance. Although SOD, CAT, and GSH remained unchanged, their overall trend suggests early compromise of antioxidant defense that may become more evident with longer or higher exposure. Consistently, Li_2CO_3-treated rat hearts showed increased MDA and reduced GPx, SOD, and CAT activities [46], while Pb exposure elevated MDA and lowered TAC and GPx [47]. Lithium may disrupt mitochondrial redox potential [46], and lead can inactivate antioxidant enzymes via sulfhydryl binding. Elevated NO may reflect iNOS induction secondary to inflammatory and oxidative signaling.

mRNA expression levels of *nrf$_2$, bax, and bcl2* in the heart

Compared to the Li and Pb-treated groups, the Li + Pb-treated group exhibited significantly downregulated *nrf2* mRNA expression levels. In the heart, *bax* mRNA expression was significantly upregulated in the Li + Pb group as compared to the Pb group. Meanwhile, *bcl-2* mRNA levels were significantly elevated in the Pb-exposed group compared to controls. Compared to the Li and Pb-treated groups, the Li + Pb-treated group exhibited significantly downregulated *bcl-2* mRNA expression levels. The *bcl-2* mRNA expression levels were also significantly upregulated in the Pb compared to the Li. The *nrf$_2$, bax, and bcl2* mRNA expression levels in the heart of the rat are graphically shown in Figure 2. mRNA expression (fold changes; $2^{-\Delta\Delta Ct}$) in the heart of male rats exposed to lithium carbonate and lead acetate depicted to Table 6.

Co-exposure to Li and Pb markedly disrupted antioxidant and apoptotic gene expression, indicating greater oxidative stress and apoptosis than individual exposures. Pb alone appeared to induce a stronger protective response than Li. The elevated *bax/bcl-2*

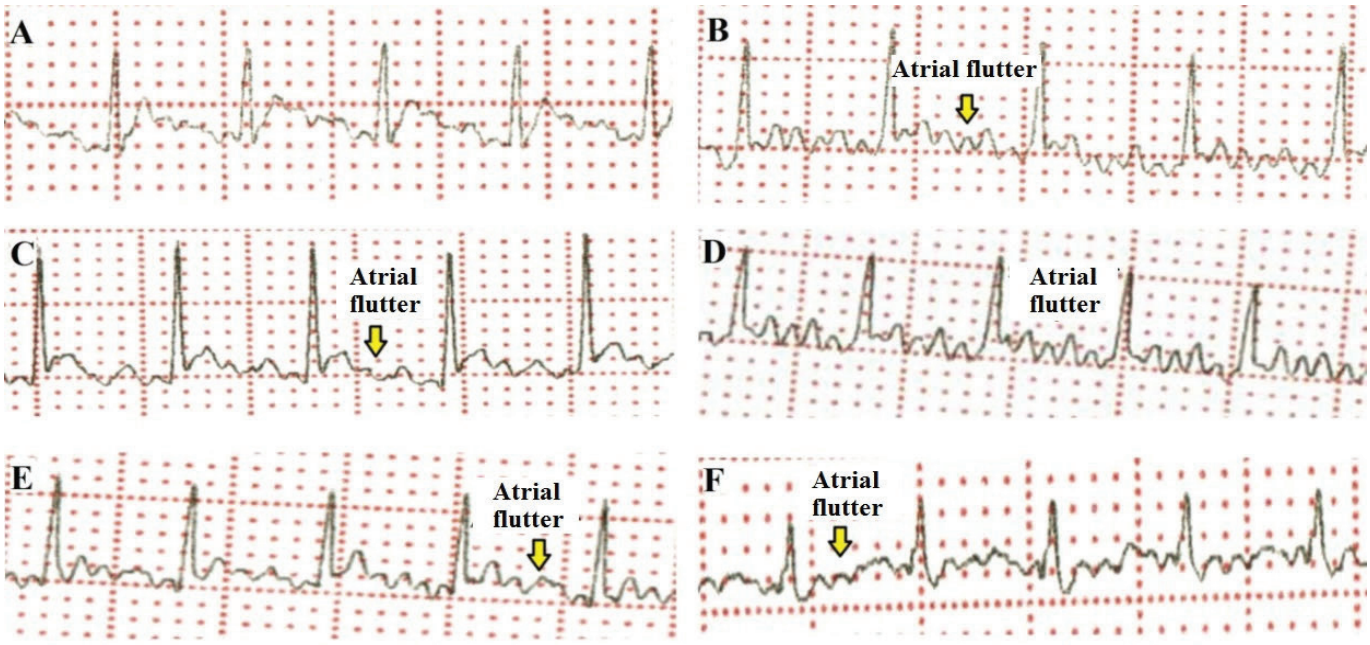

Fig. 1. ECG abnormalities in male rats exposed to lithium carbonate and lead acetate. [(A) Control, (B, C) Li, (D) Pb, (E, F) Li + Pb].

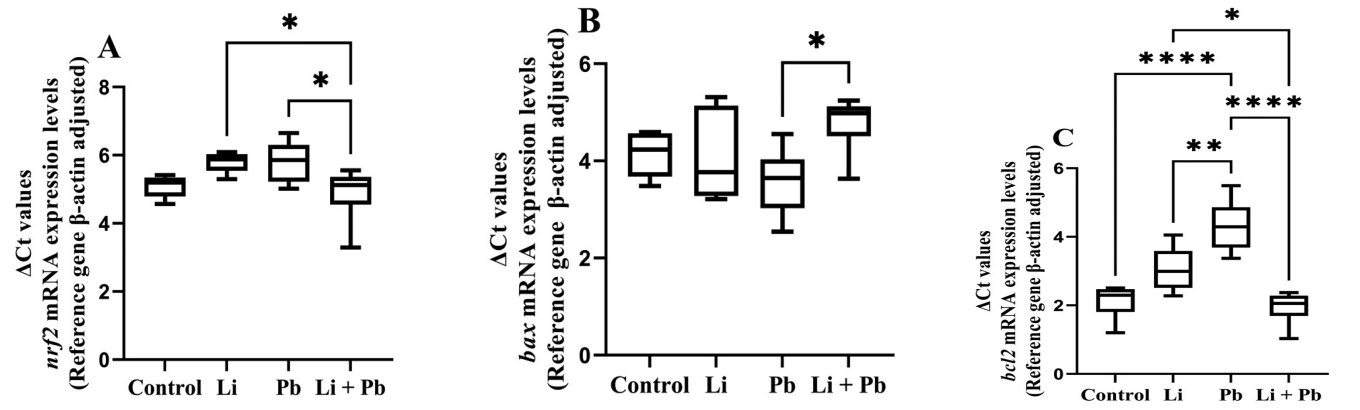

Fig. 2. The *nrf*$_2$, *bax, and bcl-2* mRNA expression levels in the heart of male rats exposed to lithium carbonate and lead acetate. [(A, B, C): Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where * indicates $p<0.05$, ** indicates $p<0.01$, **** indicates $p<0.001$].

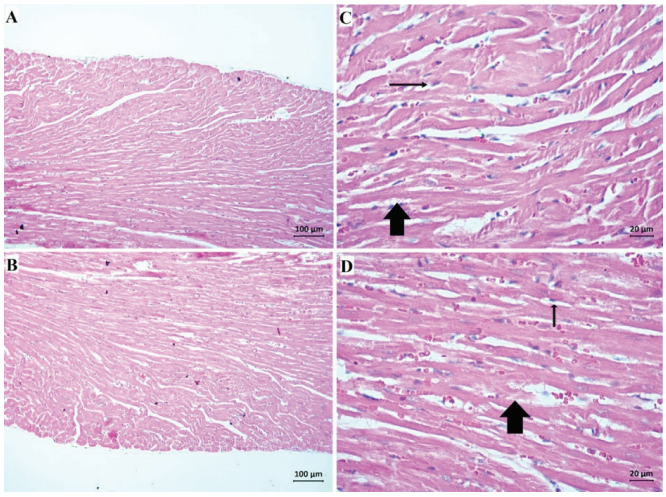

Fig. 3. Representative photographs of the microscopic view of the heart of male rats from the control group showing normal architecture of myocardium, normal arrangement of cardiac muscle fibres (thick arrow), and cardiac myocytes (thin arrow). (A, B: 100x; C, D: 400x; H and E staining).

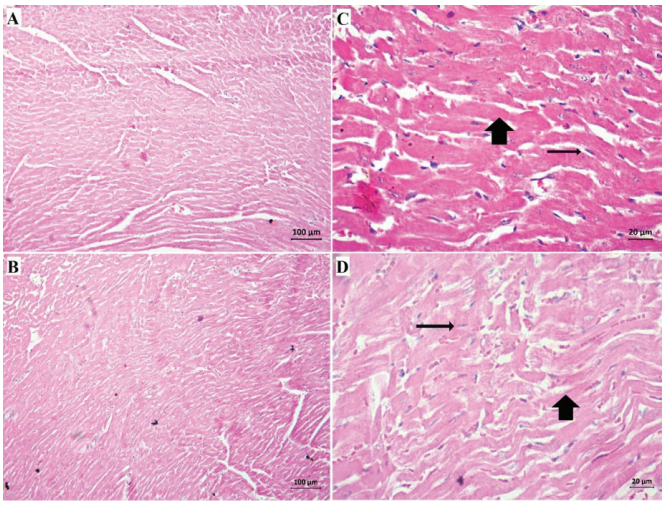

Fig. 4. Representative photographs of the microscopic view of the heart of male rats from the Li group showing normal architecture of myocardium, normal arrangement of cardiac muscle fibres (thick arrow), and cardiac myocytes (thin arrow). (A, B: 100x; C, D: 400x; H and E staining).

ratio reflects enhanced intrinsic apoptotic signaling. Oxidative stress–driven nrf2 activation normally promotes antioxidant gene expression [68], with nuclear translocation occurring under *Keap1* dissociation [69], though chronic ROS may blunt this response. Our results agree with studies reporting *nrf2* upregulation [43, 44] and conversely, [42] observed reduced *bcl-2* expression alongside increased *bax* levels in Pb-treated rats. Similarly, [70] and [71] demonstrated Li- and Pb-induced downregulation of *bcl-2* and *nrf2*, respectively. The *bax/bcl-2* shift confirms mitochondrial-mediated cardiomyocyte apoptosis; slight *bcl-2* upregulation in Pb-treated rats may reflect a transient adaptation that is suppressed under co-exposure due to lithium's inhibition of anti-apoptotic signaling [72, 73].

Histopathology

The microscopic view of the heart of rats from the control group showed normal architecture of myocardium, normal arrangement of cardiac muscle fibers, and cardiac myocytes (Fig. 3). No notable histopathological alterations were observed in the cardiac tissues of any treatment group relative to the control group (Fig. 4, 5, 6).

Despite the biochemical and molecular alterations, no significant gross pathological changes were observed

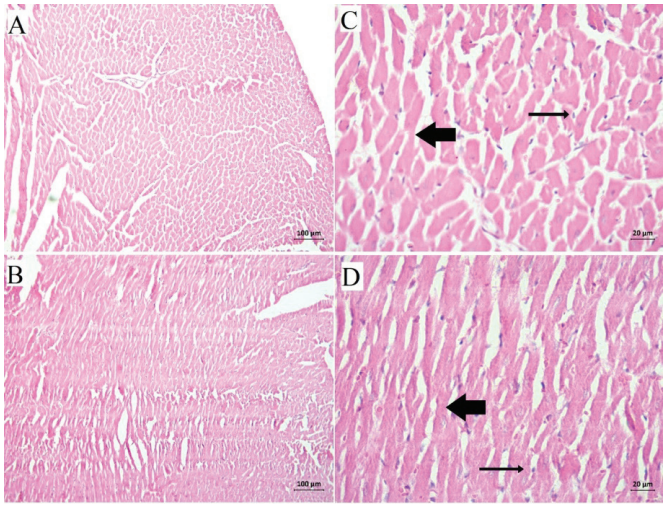

Fig. 5. Representative photographs of the microscopic view of the heart of male rats from the Pb group showing normal architecture of myocardium, normal arrangement of cardiac muscle fibres (thick arrow), and cardiac myocytes (thin arrow). (A, B: 100x; C, D: 400x; H and E staining).

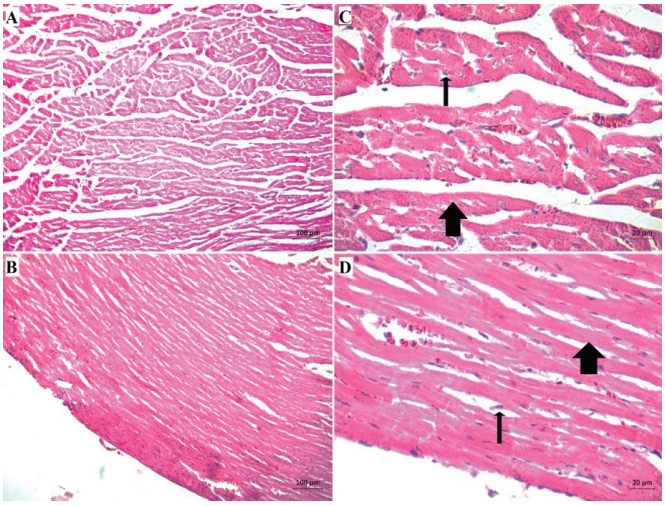

Fig. 6. Representative photographs of the microscopic view of the heart of male rats from Li + Pb groups showing normal architecture of myocardium, normal arrangement of cardiac muscle fibres (thick arrow), and cardiac myocytes (thin arrow). (A, B: 100x; C, D: 400x; H and E staining).

in the heart across any treatment group. Histological analysis showed normal cardiac architecture in all groups, including the control, with well-organized myocardium, properly aligned cardiac muscle fibers, and intact myocytes. This suggests that under the experimental exposure levels, Li and Pb did not induce overt cardiac histopathology. These results contrast with previous studies where Li_2CO_3 or Pb exposure led to evident myocardial fiber separation, necrosis, edema, and inflammatory infiltration [14, 46, 59]. Lead-induced histopathological changes such as myocyte vacuolization, necrosis, and inflammatory infiltration have been reported in longer-term exposures [18], implying that our 28-day period may reflect an early injury window.

The consistent pattern across clinical signs, ECG abnormalities, redox markers, and gene expression supports lithium–lead synergism in cardiac injury. Individually, each agent disturbs cardiac physiology, but their combined effect overwhelms adaptive mechanisms, causing electrophysiological instability and early apoptotic activation. This is evident from the co-exposed group's pronounced ECG changes, oxidative imbalance, and strong pro-apoptotic gene expression. Furthermore, correlations between increased MDA/NO and bax expression, and decreased TAC and nrf2 levels, highlight the link between oxidative stress and apoptosis, suggesting that ROS not only damage biomolecules but also trigger apoptotic signaling.

Co-exposure to lithium and lead is becoming more common in industrial regions due to widespread battery, electronic, and paint/pipe sources. Although cardiac histology appeared normal, biochemical and molecular alterations indicated early oxidative stress and apoptosis. These findings suggest additive or synergistic toxicity, emphasizing the need for holistic mixed-exposure assessments and early molecular biomarkers. Environmental monitoring of soil and water for multiple toxicants should inform improved regulatory limits. Future research must explore longer exposure periods, dose dependency, and detailed mechanisms to enhance translational relevance.

CONCLUSION

Overall, combined exposure to Li and Pb resulted in more severe toxic effects than individual exposures. Significant alterations in cardiac electrophysiology, biochemical, physiological, and organ function were observed, particularly in the Li + Pb group. These findings underscore the heightened risk associated with co-exposure and emphasize the importance of evaluating combined toxicants in environmental and occupational health assessments.

ACKNOWLEDGEMENT

All the authors are highly thankful to Dr. B. J. Trangadia, Associate Professor, Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India, for help during the study.

REFERENCES

1. Jarup L. Hazards of heavy metal contamination. Br Med Bull. 2003; 68: 167-182, DOI:10.1093/bmb/ldg032.

2. Luoma SN, Rainbow PS. Metal contamination in aquatic environments: science and lateral management. J Fish Biol. 2008; 7: 129-141.

3. Livingstone DR. Oxidative stress in aquatic organisms in relation to pollution and aquaculture. Rev Med Vet (Toulouse). 2003; 154: 427-430.

4. D'souza HS, Dsouza SA, Menezes G, Venkatesh T. Diagnosis, evaluation, and treatment of lead poisoning in general population. Indian J Clin Biochem. 2011; 26: 197-201, DOI: 10.1007/s12291-011-0122-6.

5. Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN. Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol. 2014; 7: 60-72, DOI: 10.2478/intox-2014-0009.

6. Choubey PK, Chung KS, Kim MS, Lee JC, Srivastava RR. Advance review on the exploitation of the prominent energy-storage element Lithium. Part II: From sea water and spent lithium-ion batteries. Miner Eng. 2017; 110: 104-121, DOI: 10.1016/j.mineng.2017.04.008.

7. Schrauzer GN. Lithium: occurrence, dietary intakes, nutritional essentiality. J Am Coll Nutr. 2002; 21: 14-21, DOI: 10.1080/07315724.2002.10719188.

8. Weiner ML. The human dose response to lithium from trace to toxic levels. In: Lithium in medicine and biology. Carnforth: Marius Press; 1993; 15-25.

9. Lagerkvist BJ, Lindell B. The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals: 131. Lithium and lithium compounds. 2002; Available from: http://hdl.handle.net/2077/4277](http://hdl.handle.net/2077/4277.

10. Trieff NM, Frey SM, Rao MS, Bunce HI, Herman B. Analysis for lithium in Texas drinking waters. 1973.

11. Casado M, Aragon MC, Gimémez C. Determination of monoamines in rat brain regions after chronic administration of lithium. Neurochemical Res. 1989; 14: 905-908, DOI: 10.1007/BF00964822.

12. Sahin O, Sulak O, Yavuz Y, Uz E, Eren I, *et al*. Lithium-induced lung toxicity in rats: the effect of caffeic acid phenethyl ester. Pathol. 2006; 38: 58-62, DOI: 10.1080/00313020500464904.

13. Ostadhadi S, Norouzi-Javidan A, Nikoui V, Zolfaghari S, Moradi A, *et al*. Nitric oxide involvement in additive antidepressant-like effect of agmatine and lithium in mice forced swim test. Psychiatry Res. 2018; 266: 262-268, DOI: 10.1016/j.psychres.2018.03.010.

14. Vijaimohan K, Devi CS, Mallika J. Chemoprotective effect of sobatum against lithium-induced oxidative damage in rats. J Young Pharm. 2010; 2: 68-73, DOI: 10.4103/0975-1483.62217.

15. Khairova R, Pawar R, Salvadore G, Juruena MF, *et al*. Effects of lithium on oxidative stress parameters in healthy subjects. Mol Med Rep. 2012; 5: 680-682, DOI: 10.3892/mmr.2011.732.

16. Kielczykowska M, Kocot J, Lewandowska A, Zelazowska R, Musik I. The protective influence of selenium on oxidant disturbances in brain of rats exposed to lithium. Physiol Res. 2015; 64: 739, DOI:10.33549/physiolres.932910.

17. Nciri R, Allagui MS, Bourogaa E, Saoudi M, Murat JC *et al*. Lipid peroxidation, antioxidant activities and stress protein (HSP72/73, GRP94) expression in kidney and liver of rats under lithium treatment. J Physiol Biochem. 2012; 68: 11-18, DOI: 10.1007/s13105-011-0113-3.

18. Oktem F, Ozguner F, Sulak O, Olgar S, Akturk O, *et al*. Lithium-induced renal toxicity in rats: protection by a novel antioxidant caffeic acid phenethyl ester. Mol Cell Biochem. 2005; 277: 109-115, DOI: 10.1007/s11010-005-5426-5.

19. Toghiani T, Gholami M, Zendedel A, Assadollahi V. The effects of low-dose lithium carbonate on the spermatogenic parameter in adult male Wistar rats. J Life Sci. 2012; 9: 4360-4367.

20. Goyer R. Toxic effects of metals. In: Amdur MO, Doull JD, Klaassen CD, editors. Casarett and Doull's Toxicology. 4th edn. New York: Pergamon Press, 1991; 623-680.

21. Godwin HA. The biological chemistry of lead. Current Opin Chemic Biol. 2001; 5: 223-227, DOI: 10.1016/s1367-5931(00)00194-0.

22. Smith DR, Flegal AR. Lead in the biosphere: recent trends. Ambio. 1995; 24: 21-23, https://www.jstor.org/stable/4314280.

23. Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med. 1995; 18: 321-336, DOI: 10.1016/0891-5849(94)00159-h.

24. Moore MR. Haematological effects of lead. Sci Total Environ. 1988; 71: 419-431, DOI: 10.1016/0048-9697(88)90214-8.

25. Smith AJ, Smith K. Guidelines for humane

education: alternatives to the use of animals in teaching and training. Altern Lab Anim. 2004; 32: 29-39, DOI: 10.1177/026119290403201s07.

26. Vediya SD, Patel SS. Contamination of toxic metals in Vastral lake, Ahmadabad, Gujarat, India. Int J Pharm Life Sci. 2011; 2: 1174-1176.

27. Patel SS, Vediya SD. A studies of toxic metals in Sarkhej lake water, Ahmedabad, Gujarat, India. Int J Pharm Life Sci. 2012; 3: 1985-1986.

28. Amari T, Ghnaya T, Abdelly C. Nickel, cadmium and lead phytotoxicity and potential of halophytic plants in heavy metal extraction. South Afric J Botany. 2017; 111: 99-110, DOI: 10.1016/j.sajb.2017.03.011.

29. Rahman S, Sultana S. Chemopreventive activity of glycyrrhizin on lead acetate mediated hepatic oxidative stress and its hyperproliferative activity in Wistar rats. Chem Biol Interact. 2006; 160: 61-69, DOI: 10.1016/j.cbi.2005.12.003.

30. Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Current Top Medic Chemist. 2001; 1: 529-539, DOI: 10.2174/1568026013394831.

31. Barbosa F Jr, Tanus-Santos JE, Gerlach RF, Parsons PJ. A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. Environ Health Perspect. 2005; 113: 1669-1674, DOI: 10.1289/ehp.7917.

32. Flora G, Gupta D, Tiwari A. Toxicity of lead: a review with recent updates. Interdiscip. Toxicol. 2012; 5: 47-58, DOI: 10.2478/v10102-012-0009-2.

33. Andjelkovic M, Buha Djordjevic A, Antonijevic E, Antonijevic B, Stanic M, *et al*. Toxic effect of acute cadmium and lead exposure in rat blood, liver, and kidney. Internati J Environment Res Public Health. 2019; 16: 274, DOI: 10.3390/ijerph16020274.

34. Dapul H, Laraque D. Lead poisoning in children. Adv Pediatr. 2014; 61: 313-333, DOI: 10.1016/j.yapd.2014.04.004.

35. Samarghandian S, Azimi-Nezhad M, Farkhondeh T, Samini F. Anti-oxidative effects of curcumin on immobilization-induced oxidative stress in rat brain, liver and kidney. Biomed Pharmacother. 2017; 87: 223-229, DOI: 10.1016/j.biopha.2016.12.105.

36. Luo H, Li J, Song B, Zhang B, Li Y, *et al*. The binary combined toxicity of lithium, lead, and manganese on the proliferation of murine neural stem cells using two different models. Environ Sci Pollut Res. 2023; 30: 5047-5058, DOI: 10.1007/s11356-022-22433-x.

37. Cunha M, Cruz I, Pinto J, Benito D, Ruiz P, *et al.* The influence of temperature on the effects of lead and lithium in *Mytilus galloprovincialis* through biochemical, cell and tissue levels: Comparison between mono and multi-element exposures. Sci Total Environ. 2023; 902: 165786, DOI: 10.1016/j.scitotenv.2023.165786.

38. Liu B, Zhang H, Tan X, Yang D, Lv Z, *et al*. GSPE reduces lead-induced oxidative stress by activating the Nrf2 pathway and suppressing miR153 and GSK-3β in rat kidney. Oncotarget. 2017; 8: 42226-42237, DOI: 10.18632/oncotarget.15033.

39. Shi X, Fu L. Piceatannol inhibits oxidative stress through modification of Nrf2-signaling pathway in testes and attenuates spermatogenesis and steroidogenesis in rats exposed to cadmium during adulthood. Drug Des Dev Ther. 2019; 13: 2811-2824, DOI: 10.2147/DDDT.S198444.

40. Heidari R, Behnamrad S, Khodami Z, Ommati MM, Azarpira N, *et al.* The nephroprotective properties of taurine in colistin-treated mice is mediated through the regulation of mitochondrial function and mitigation of oxidative stress. Biomed Pharmacother. 2019; 109: 103-111, DOI: 10.1016/j.biopha.2018.10.093.

41. Zhang Y, Wang Y, Runliu YU, Zhang S, Wu Z. Effects of heavy metals Cd^{2+}, Pb^{2+}, Zn^{2+} on DNA damage of loach *Misgurnus anguillicaudatus*. Front Biol China. 2008; 3: 50-54, DOI: 10.1007/s11515-008-0012-3.

42. Al-Quraishy S, Dkhil MA, Ibrahim SR, Moneim AEA. Neuroprotective potential of *Indigofera oblongifolia* leaf methanolic extract against lead acetate-induced neurotoxicity. Neural Regen Res. 2016; 11: 1797-1803, DOI: 10.4103/1673-5374.194749.

43. Abbaszadeh S, Yadegari P, Imani A, Taghdir M. Vitamin D3 protects against lead-induced testicular toxicity by modulating *Nrf2* and *NF-κB* gene expression in rats. Reproduct Toxicol. 2021; 103: 36-45, DOI:10.1016/j.reprotox.2021.05.008.

44. Bidanchi RM, Lalrindika L, Khushboo M, Bhanushree B, Dinata R, *et al*. Antioxidative, anti-inflammatory and anti-apoptotic action of ellagic acid against lead acetate induced testicular and hepato-renal oxidative damages and pathophysiological changes in male Long Evans rats. Environmental Pollution. 2022; 302: 119048, DOI: 10.1016/j.envpol.2022.119048.

45. Ben Saad A, Dalel B, Rjeibi I, Smida A, Ncib S, *et al*. Phytochemical, antioxidant and protective effect of cactus cladodes extract against lithium-induced liver injury in rats. Pharm Biol. 2017; 55: 516-525, DOI: 10.1080/13880209.2016.1255976.

46. Saad AB, Rjeibi I, Alimi H, Ncib S, Smida A, *et al*. Lithium induced oxidative stress and related damages in

testes and heart in male rats: the protective effects of *Malva sylvestris* extract. Biomed Pharmacother. 2017; 86: 127-135, DOI: 10.1016/j.biopha.2016.12.105.

47. Roshan VD, Assali M, Moghaddam AH, Hosseinzadeh M, Myers J. Exercise training and antioxidants: effects on rat heart tissue exposed to lead acetate. Int J Toxicol. 2011; 30: 190-196, DOI: 10.1177/1091581810392809.

48. Rahimi Balaei M, Momeny M, Babaeikelishomi R, Ejtemaei Mehr S, Tavangar SM, *et al*. The modulatory effect of lithium on doxorubicin-induced cardiotoxicity in rat. Eur J Pharmacol. 2010; 641: 193-198, DOI: 10.1016/j.ejphar.2010.05.046.

49. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974; 47: 469-474, DOI: 10.1111/j.1432-1033.1974.tb03714.x.

50. Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem. 1952; 195: 133-140, DOI: 10.1016/S0021-9258(19)50881-X.

51. Ellman G. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82: 70-77, DOI: 10.1016/0003-9861(59)90090-6.

52. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt Biochem. 1976; 72(1-2): 248-254.

53. Aebi H, Wyss SR, Scherz B, Skvaril F. Heterogeneity of erythrocyte catalase II. Isolation and characterization of normal and variant erythrocyte catalase and their subunits. European J Biochem. 1974; 48(1): 137-145, DOI: 10.1111/j.1432-1033.1974.tb03751.x.

54. Sambrook L, Russell RW. Molecular cloning: a laboratory manual. 3rd edn. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2001.

55. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 2001; 25: 402-408, DOI: 10.1006/meth.2001.1262.

56. Luna LG. Routine staining procedures: hematoxylin and eosin stains. In: Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd edn. New York: McGraw-Hill; 1968; 32-39.

57. Etling N, Levy M, Fouque F. Thyroid hormones in rats receiving increasing doses of lithium. Annales d'Endocrinologie. 1987; 48: 452-456.

58. Henry C. Lithium side-effects and predictors of hypothyroidism in patients with bipolar disorder: sex differences. J Psychiatry Neurosci. 2002; 27: 104-107.

59. Abdel Hamid OI, Ibrahim EM, Hussien MH, ElKhateeb SA. The molecular mechanisms of lithium-induced cardiotoxicity in male rats and its amelioration by N-acetyl cysteine. Hum Exp Toxicol. 2020; 39(5): 696-711, DOI: 10.1177/0960327119897759.

60. L'Abbate S, Nicolini G, Marchetti S, Forte G, Lepore E, *et al*. Lithium treatment induces cardiac dysfunction in mice. Int J Mol Sci. 2023; 24: 15872, DOI: 10.3390/ijms242115872.

61. Ahmed MA, Hassanein KM. Cardio protective effects of *Nigella sativa* oil on lead induced cardio toxicity: Anti-inflammatory and antioxidant mechanism. J Physiol Pathophysiol. 2013; 4: 72-80, DOI: 10.5897/JPAP2013.0083.

62. Ungvary G, Morvai V, Szakmáry É. Haemodynamic effects of lead (II) acetate, ethanol, and their combination in rats. Int Arch Occup Environ Health. 2002; 75: 45-53, DOI: 10.1007/s00420-002-0356-y.

63. Reza B, Ali N, Azhdar H, Alireza A, Ali K. Effects of low-level lead exposure on blood pressure and function of the rat isolated heart. Indian J Pharmacol. 2008; 40: 69-72, DOI: 10.4103/0253-7613.41041.

64. Klinova SV, Minigalieva IA, Protsenko YL, Sutunkova MP, Gurvich VB, *et al*. Changes in the cardiotoxic effects of lead intoxication in rats induced by muscular exercise. Int J Mol Sci. 2022; 23: 4417-4434, DOI: 10.3390/ijms23084417.

65. Ferreira de Mattos G, Costa C, Savio F, Alonso M, Nicolson GL. Lead poisoning: acute exposure of the heart to lead ions promotes changes in cardiac function and Cav1.2 ion channels. Biophys Rev. 2017; 9: 807-825, DOI: 10.1007/s12551-017-0303-5.

66. Alda M. Lithium in the treatment of bipolar disorder: pharmacology and pharmacogenetics. Mol Psychiatry. 2015; 20: 661-670, DOI: 10.1038/mp.2015.4.

67. Kumar Singh P, Kumar Singh M, Singh Yadav R, Kumar Dixit R, Mehrotra A, *et al*. Attenuation of lead-induced neurotoxicity by omega-3 fatty acid in rats. Ann Neurosci. 2018; 24: 221-232, DOI: 10.1159/000481808.

68. Osburn WO, Kensler TW. Nrf2 signaling, an adaptive response pathway for protection against environmental toxic insults. Mutat Res Rev Mutat Res. 2008; 659: 31-39, DOI: 10.1016/j.mrrev.2007.11.006.

69. Dai G, Vaughn S, Zhang Y, Wang ET, Garcia-Cardena G, Gimbrone MA Jr. Biomechanical forces in atherosclerosis-resistant vascular regions regulate endothelial redox balance

via PI3K/Akt-dependent activation of Nrf2. Circulation Res. 2007; 101: 723-733, DOI: 10.1161/circresaha.107.152942.

70. Erbaş E, Gelen V, Öztürk E. Therapeutic effect of d-carvone on inflammation, apoptosis, and cell damage in lithium-induced liver injury model in rats. Turkish J Vet Res. 2024; 8: 95-101, DOI: 10.47748/tjvr.1428357.

71. Albarakati AJA, Baty RS, Aljoudi AM, Habotta OA, Elmahallawy EK, *et al*. Luteolin protects against lead acetate-induced nephrotoxicity through antioxidant, anti-inflammatory, anti-apoptotic, and Nrf2/HO-1 signaling pathways. Mol Biol Reports. 2020; 47: 2591-2603, DOI: 10.1007/s11033-020-05346-1.

72. Bashandy MM, Saeed HE, Ahmed WM, Ibrahim MA, Shehata O. Cerium oxide nanoparticles attenuate the renal injury induced by cadmium chloride via improvement of the NBN and Nrf2 gene expressions in rats. Toxicol Res. 2022; 11(2): 339-347.

73. Yousefvand S, Keshavarzi Z, Dolatshad H, Vafaee F, Mahmoudabady M, *et al*. Effects of prolactin on movement disorders and APOE, GFAP, and PRL receptor gene expression following intracerebral hemorrhage in rats. Iran J Basic Med Sci. 2021; 24(12): 1709-1716, DOI: 10.22038%2FIJBMS.2021.58176.12927.

Cite this article as: Patel HR, Patel UD, Prajapati MD,. Humbal BR, Patel JP, Fefar DT. Early cardiac damage following single and combined exposure to lithium carbonate and lead acetate in rats: a toxicological perspective. Explor Anim Med Res. 2025; 15(2), DOI: 10.52635/eamr/15.2.206-218.